Sporogenesis in Pteris cretica with Special Reference to the Cytoplasmic Inclusions

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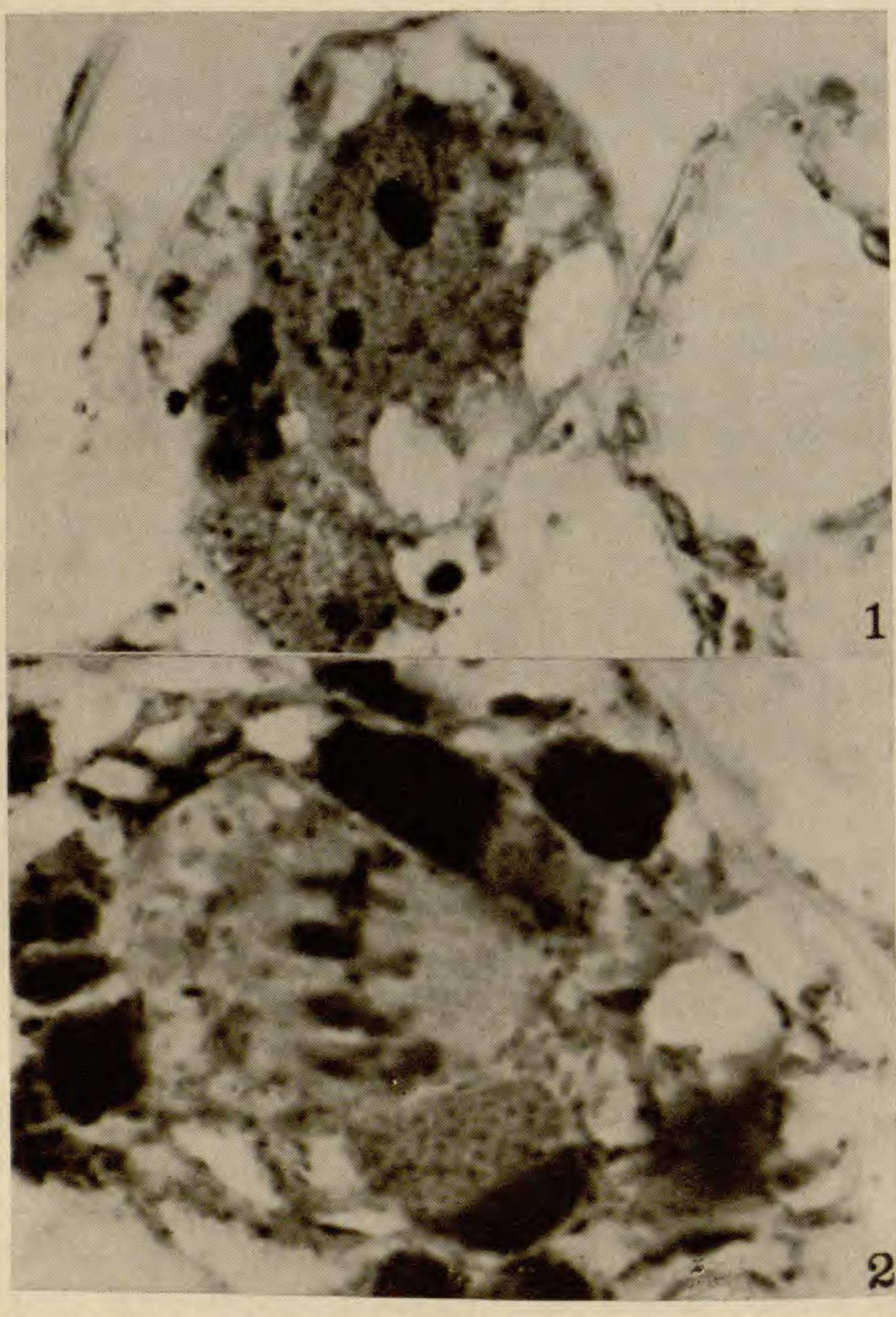
INTRODUCTION.—Studies dealing with the cytoplasmic inclusions during sporogenesis in polypodiaceous ferns have indicated that in at least three species, *Nephrodium molle* (Senjaninova 1927), *Onoclea sensibilis* (Marengo 1949) and *Polypodium virginianum* (Marengo 1959), cytokinesis of the spore mother cell results in the formation of a quartet of tetragonally arranged, and upon maturity, monolete, spores.

Adiantum hispidulum, a polypodiaceous fern bearing trilete spores was similarly investigated (Marengo 1962). In this species the cytoplasmic inclusions preserved by mitochondrial fixatives were found to be arranged, prior to cytokinesis, in a pattern of six planes marking the sites of the internal walls of the four tetrahedrally arranged spores arising from the spherical spore mother cell. The cytokinetic basis of tetrahedral symmetry in this species was thus found to be identical to that described in *Osmunda regalis* (Marengo 1954).

On the basis of the cytokinetic similarity of the maturation divisions of these two unrelated species, both producing trilete, tetrahedrally symmetrical spores, it was suggested that further studies might show in other species a similar sequence of events in meiosis, and thus possibly reflect a pattern of cytokinesis common to the production of all trilete spores, regardless of the taxonomic position of the species considered.

Through the courtesy of Dr. William Steere, material of *Pteris cretica* was made available at the New York Botanical Garden. This member of the Polypodiaceae produces typically and obviously trilete spores. It is the major object of this paper to describe the cytokinetics of the maturation divisions in this species and to compare them with previously described processes in *Osmunda regalis* and *Adiantum hispidulum*, and to clarify the suggestion that a common process and sequence hold for the production of all trilete spores.

MATERIALS AND METHODS.—Fertile leaf margin pieces under 5 mm in length were excised from greenhouse plants and im-



FIGS. 1-2. EARLY STAGES OF SPORANGIAL DEVELOPMENT. × 1578. FIG. 1. EARLY SPORANGIUM SHOWING INTERPHASE OF INITIAL SPOROGENOUS CELL PRIOR TO TAPETAL CELL FORMATION. FIG. 2. METAPHASE OF CENTRAL SPOROGENOUS CELL SURROUNDED BY EARLY TAPETUM.

mediately placed in fixative. To preserve the cytoplasmic inclusions, the material was fixed from twelve to twenty-four hours in a solution freshly prepared as follows (Huseby 1946): 10% Commercial formalin, 100 ml; Normal sodium hydroxide, 1 ml; Pyrogallol, 7 g.

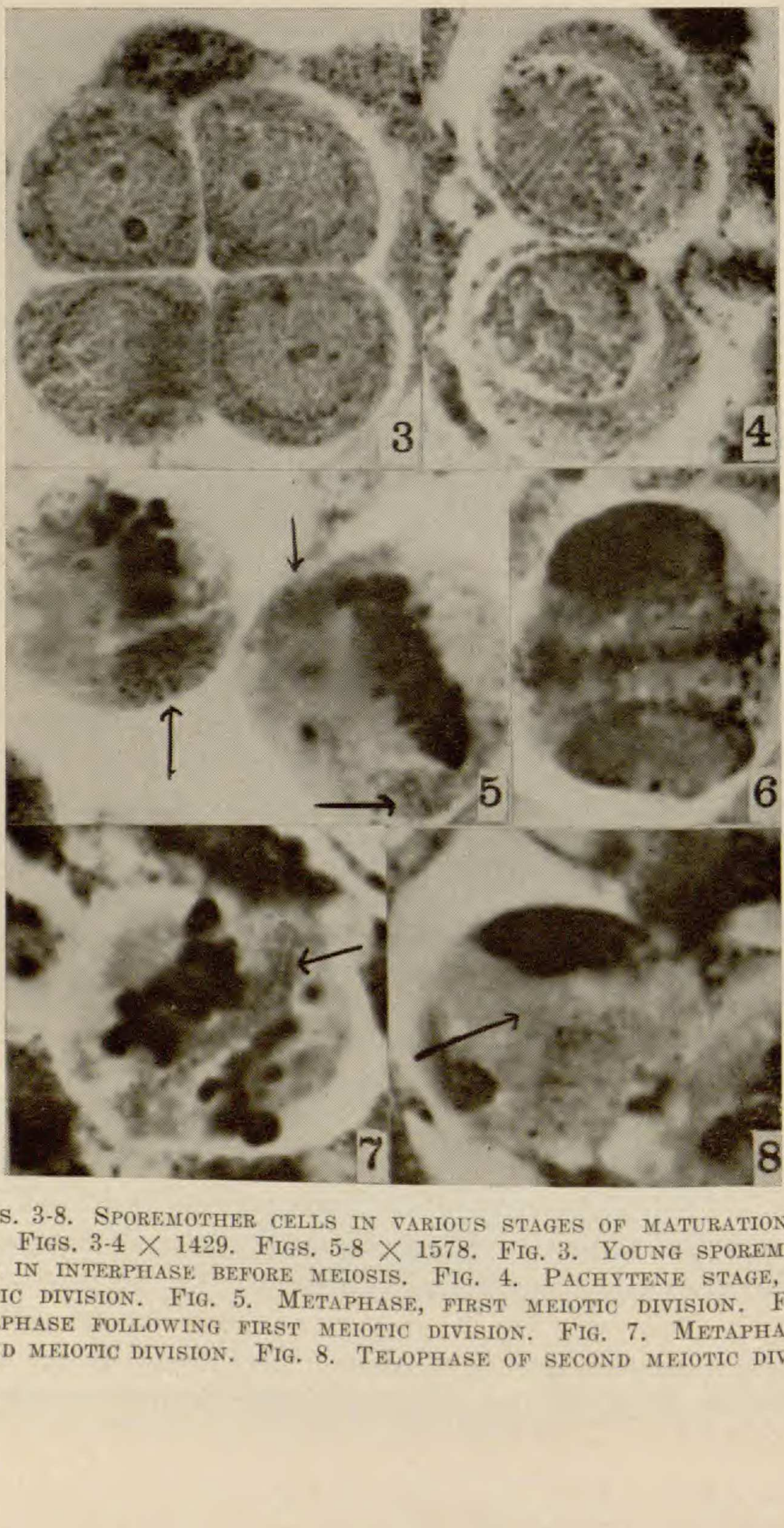
Pieces were rinsed briefly in running water, dehydrated with isopropyl alcohol and embedded in 56°–58° Fisher Tissuemat. Sections three microns in thickness were cut without difficulty.

Sections were mordanted twenty-four hours in 2 percent ferric alum, stained twenty-four hours in 0.5 percent hematoxylin and differentiated with microscopic observation in 2 percent ferric alum. Following a thirty-minute wash in running tap water, slides were dehydrated in isopropyl alcohol, counterstained with Orange G in clove oil, cleared in xylene and mounted in gum damar. Material similarly fixed and sectioned was stained with acid fuchsin and methyl green.

Observations and photomicrographs were made with a Bausch and Lomb Dynazoom microscope with a 97X achromatic objective; 4″ × 5″ negatives were obtained using a Brinkmann Model "U" photomicrographic camera with Kodak Royal Pan sheet film. Negatives were obtained also with Bausch and Lomb 35 mm photomicrographic camera, using Panatomic-X film.

OBSERVATIONS.—The mature spore of *Pteris cretica* bears a characteristic tri-radiate scar which marks the internal edges of the triangular spherical pyramid which originated as one of the four tetrahedrally symmetrical spores in the young quartet arising by meiosis from the spherical spore mother cell (Fig. 12). Its original pyramidal shape is obscured by enlargement and spore coat formation.

The early development of the sporangium is of the usual leptosporangiate type. A large sporogenous cell is separated at an early stage from the cells to become the sporangial wall (Fig. 1). This cell (Fig. 2) soon gives rise to tapetal cells and then to a central group of sporogenous cells. Prior to the maturation divisions, the sporogenous cells assume the rounded-



FIGS. 3-8. SPOREMOTHER CELLS IN VARIOUS STAGES OF MATURATION DIVISIONS FIGS. 3-4 × 1429. FIGS. 5-8 × 1578. FIG. 3. YOUNG SPOREMOTHER CELLS IN INTERPHASE BEFORE MEIOSIS. FIG. 4. PACHYTENE STAGE, FIRST MEIOTIC DIVISION. FIG. 5. METAPHASE, FIRST MEIOTIC DIVISION. FIG. 6. INTERPHASE FOLLOWING FIRST MEIOTIC DIVISION. FIG. 7. METAPHASE OF SECOND MEIOTIC DIVISION. FIG. 8. TELOPHASE OF SECOND MEIOTIC DIVISION.

up shape of the young spore mother cell (Fig 3). The mitochondria of the spore mother cell in the interphase preceding maturation are small, granular or rod-like, and of uniform distribution throughout the cytoplasm.

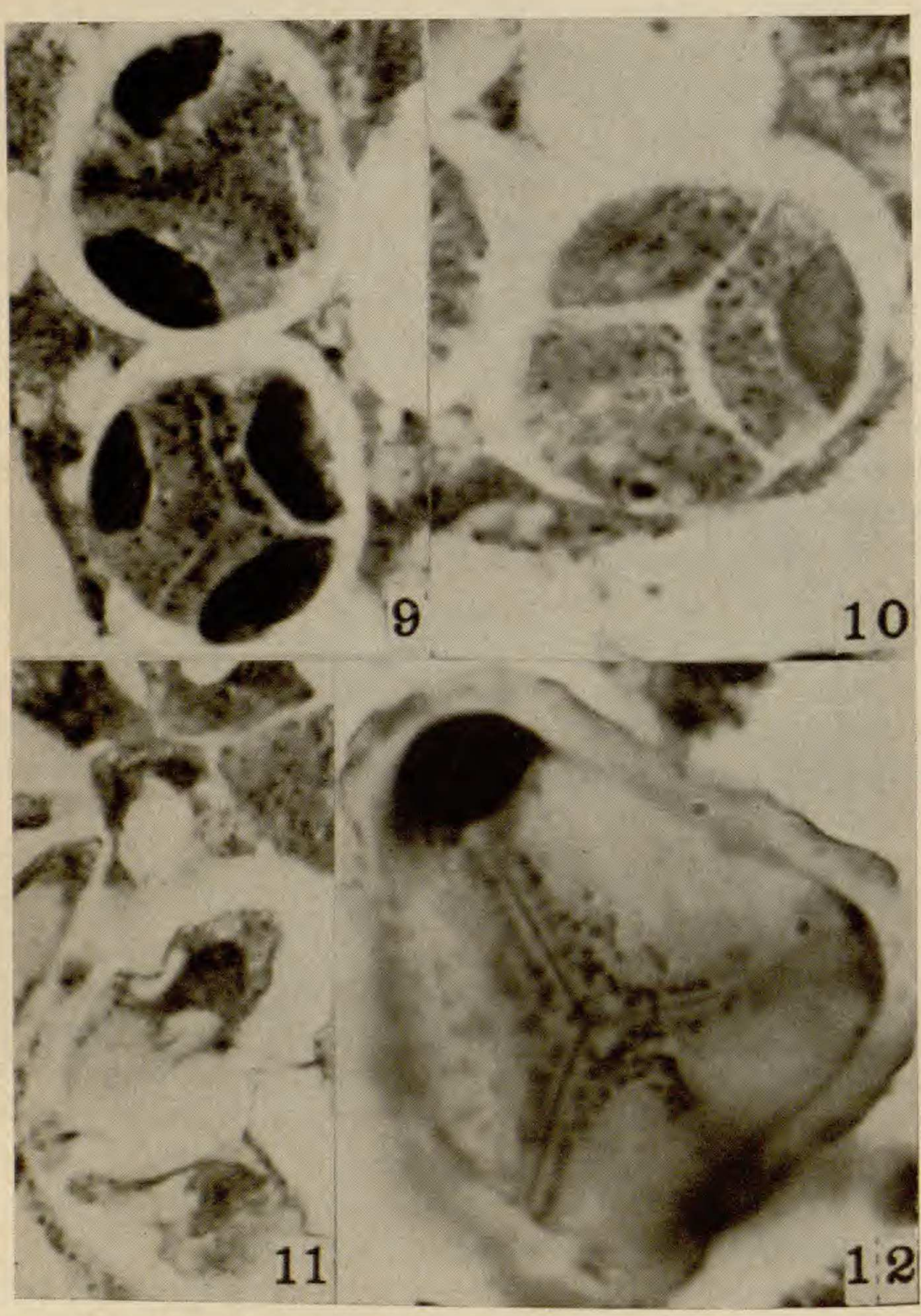
At the pachytene stage of the first meiotic prophase, the chromosomes are polarized into a "bouquet," with an accompanying polarization of the cytoplasmic inclusions. A small concentration is at the base of the bouquet and a larger mass at the opposite pole of the spore mother cell (Fig. 4).

At the metaphase of the first meiotic division, the cytoplasmic inclusions are almost completely localized in a ring or torus surrounding the metaphase plate. In a pole-to-pole section of this stage, this ring appears in cross section as a mass of granules at the edge of the metaphase plate (Fig. 5). As the first division proceeds, this ring of granules moves centrally into the spindle area. At the time of interphase following the first division this ring has been converted into a compact, flat disc interposed between the nuclei (Fig. 6). There is no indication of cytokinesis up to this point.

The first indication of cytokinesis appears at the metaphase of the second division. Favorable sections at this stage show the mitochondrial plate with a two-layered structure, suggesting that a cell plate has formed within it, although light microscopy has not as yet verified its presence (Fig. 7). By late telophase of the second division, a cell plate can be detected in favorable sections longitudinal to one of the second division spindles. This cell plate is formed completely independent of the mitochondrial localization which at this time assumes a folded structure, showing as a "V" in cross section (Fig. 8).

As the second maturation division is completed, the cytoplasmic inclusions are regularly arranged along all the internal walls of the newly-formed spores (Fig. 9). Prior to spore enlargement and quartet separation, these granules lose their localization and assume a uniform distribution in the cytoplasm of the young spore (Fig. 10).

It is difficult to properly fix and stain the young enlarging



FIGS. 9-12. SPORES FOLLOWING COMPLETION OF MEIOSIS. FIGS. 9, 11, 12 × 1429. FIG. 10 × 1578. FIG. 9. INTERPHASE IMMEDIATELY FOLLOWING COMPLETION OF SECOND MEIOTIC DIVISION. FIG. 10. SLIGHTLY OLDER QUARTET THAN FIG. 9. FIG. 11. ENLARGING SPORES. FIG. 12. MATURE SPORE SECTIONED AND SHOWING INTERNAL VIEW OF TRILETE SCAR.

spore. This difficulty may be associated with vacuole formation and spore coat deposition (Fig. 11). Stained sections through mature spores show the nucleus at one of the ends of the trilete scar, and numerous large granules along the scar itself (Fig. 12). The precise nature of these granules in the mature spore, and their genetic continuity with the mitochondria of the spore mother cell have yet to be established.

DISCUSSION.—The behavior of the cytoplasmic inclusions during the first meiotic division in *Pteris cretica* appears to be essentially like that observed previously in *Onoclea sensibilis* and *Adiantum hispidulum*. The arrangement of the late prophase chromosomes into a bouquet is accompanied by polarization of cytoplasmic inclusions comparable to the same stage in the other two species mentioned. The localization of granules into a compact plate between the interphase nuclei resulting from the first division is observed in the same stage in *Onoclea sensibilis* and *Adiantum hispidulum* as well as in *Osmunda regalis*.

In the two trilete species, *Osmunda regalis* and *Adiantum hispidulum*, there is no indication of cytokinesis or cell plate formation prior to the interphase after the second division, at which point the mitochondrial plates separating the nuclei are in two distinct layers. In *Pteris cretica* cytokinesis of the first meiotic division has started by the time the second division has reached metaphase. This is suggested by the two-layered structure of the mitochondrial disc formed following the first division. The completion of cytokinesis in *P. cretica* is accomplished by cell plate formation independent of any mitochondrial localization, and would suggest that in this species at least, this process and its relation to the establishment of tetrahedral symmetry of the spore quartet is not related to the distribution of the cytoplasmic inclusions during meiosis. In *Osmunda regalis*, prior to cytokinesis, the future internal walls of the spores appear to be delineated by mitochondrial plates. This suggests an inherent tetrahedral symmetry of the spore mother cell. The findings in *Pteris cretica* appear to support this idea,

except that this symmetry is established independent of and unrelated to the cytoplasmic inclusions preserved by mitochondrial fixatives.

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Florida Strap Ferns and Their Culture

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The fern flora of the state of Florida is a remarkable one, the largest of that of any of the United States, insofar as I am aware. Of this assemblage, one of the most fascinating genera is that which contains the Strap Ferns, the genus *Campyloneurum*. Of the fifty or so species known to science (this genus is often included in *Polypodium*), four have made their way into our area, where one is a reasonably common indigene, while the others are definite and restricted rarities.

Campyloneurum phyllitidis is by far the most frequent and most widespread of the Strap Ferns here. Small, in *Ferns of Florida* (1931), notes it as occurring in "hammocks, lower two-thirds of the Florida peninsula and Florida keys." It is, however, not strictly an inhabitant of our marvelous hammocks,